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Prevalent Loss of Mitotic Spindle Checkpoint in Adult T-cell Leukemia Confers Resistance to Microtubule Inhibitors*

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Human T-cell leukemia virus type I (HTLV-I) is the causative agent for adult T-cell leukemia (ATL). Molecularly, ATL cells have extensive aneugenic abnormalities that occur, at least in part, from cell cycle dysregulation by the HTLV-I-encoded Tax oncoprotein. Here, we compared six HTLV-I-transformed cells to Jurkat and primary peripheral blood mononuclear cells (PBMC) in their responses to treatment with microtubule inhibitors. We found that both Jurkat and PBMCs arrested efficiently in mitosis when treated with nocodazole. By contrast, all six HTLV-I cells failed to arrest comparably in mitosis, suggesting that ATL cells have a defect in the mitotic spindle assembly checkpoint. Mechanistically, we observed that in HTLV-I Tax-expressing cells human spindle assembly checkpoint factors hsMAD1 and hsMAD2 were mislocated from the nucleus to the cytoplasm. This altered localization of hsMAD1 and hsMAD2 correlated with loss of mitotic checkpoint function and chemoresistance to microtubule inhibitors.

In vivo infection by human T-lymphotropic virus type I (HTLV-I)[1] engenders adult T-cell leukemia (ATL) in a minority of individuals after a prolonged latent period. The pathological course of ATL suggests a multistage process of transformation beginning from clonal expansion of an HTLV-I-bearing T-cell followed by the accumulation of cellular genetic lesions that likely inactivate several tumor-suppressor genes (1–7). ATL cells harbor significant clastogenic as well as aneugenic chromosomal abnormalities (8, 9). HTLV-I has been found to subvert several cellular checkpoints that guard against loss of genome integrity (10). Indeed, in a process that possibly explains aneuploidy in ATL cells, it was recently shown that the HTLV-I Tax oncoprotein inactivates the function of the human spindle assembly checkpoint protein, MAD1 (11).

Aneuploidy is seen in ~70% of all cancers. Genetic studies in yeast have implicated at least seven genes (MAD (mitotic arrest deficiency)-1, -2, -3; BUB (budding uninhibited by benomyl)-1, -2, -3; and MPS1 (monopolar spindle 1)) (12) in the mitotic spindle checkpoint, which censors against aneuploidy. These checkpoint proteins form complexes that regulate orderly chromosomal segregation and nuclear division (13–15). Interestingly, despite the high frequency of aneuploidy in human cancers, only rarely have genetic defects in mitotic checkpoint genes been found (16, 17). This suggests that events other than genetic changes may abrogate mitotic spindle checkpoint function and account for aneugenic alterations.

We have employed HTLV-I-transformed human T-cells as a model to investigate the biology of the mitotic spindle checkpoint in cancers. Here, we report the prevalent loss of mitotic spindle checkpoint in six out of six HTLV cells. We further show that this defect in mitotic checkpoint function correlated with resistance by ATL cells to MTI agents.

EXPERIMENTAL PROCEDURES

Cell Lines and Cell Cycle Analysis—MT-1, MT-4, TL-Omi, TL-Su, ILT-Hod, and C8166 are human HTLV-1-transformed T-cell lines (MT-1, MT-4, TL-Omi, TL-Su, and C8166 are IL-2-independent; ILT-Hod is IL-2-dependent). IL-2-independent cells and Jurkat cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum (RPMI-FCS). ILT-Hod was cultured in RPMI-FCS with 1 nM IL-2. HeLa and SW480 were cultured in DMEM with 10% FCS. Primary human PBMCs from anonymous normal donors were obtained from the NIH blood bank. PBMCs were activated to proliferate by treatment with 20 units/ml of recombinant human IL-2 (Roche Molecular Biochemicals) and 0.25 μg/ml phytohemagglutinin (Roche Molecular Biochemicals) in RPMI-FCS for 3 days prior to nocodazole assays.

Mitotic Index and Apoptosis Analysis—Nocodazole (Sigma) was added to medium at final concentrations of 0.1, 0.5, or 1.0 μM, as indicated. Cells were harvested at 12-h time intervals up to 36 h. After harvesting, the cells were pelleted (1500 rpm, 5 min) and washed with PBS. Cell pellets were resuspended in 50 μl of 1% formaldehyde, 0.2% glutaraldehyde. 20 μl of the cell suspension were dried onto a poly-L-lysine-coated slide, washed with PBS, and stained with PBS containing 10 μg/ml of Hoechst 33258 (Sigma) for 10 min at room temperature. Fluorescent microscopy was used to visualize viable cells arrested in mitosis. To measure the mitotic index, at least 300 cells were counted in each assay. All assays were repeated two to three times. Quantitations of apoptosis by Hoechst dye staining and by TUNEL assay (not shown) were also similarly performed. Cytogenetic analyses of cells, where reported, were performed by the Cell Culture Laboratory of the Children's Hospital of Michigan.

[^{3}H]Thymidine Incorporation—Suspension cells were incubated at 1×10^5 cells/ml. Twenty hours after the addition of nocodazole, [^{3}H]thymidine was added (10 μCi/ml). Four hours later cells were pelleted and washed with 0.5 ml of PBS, then methanol/acetic acid (3:1) was added for 15 min to fix cells, followed by two washes with 0.5 ml of methanol/acetic acid. The cell pellet was solubilized with 0.25 ml of 0.1 N NaOH and transferred to scintillation vials. After addition of 5 ml of scintillant, incorporated [^{3}H]thymidine was measured by scintillation spectroscopy.

Cell Viability Assay—Cells (5×10^4 cells/ml) were treated with nocodazole (0.5 μM) or vincristine (0.5 μM) or 300 nM of flavopiridol and harvested at indicated intervals. Cell viability was measured by a modified MTT dye reduction assay using WST-8 (2-(2-methoxy-

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[1] The abbreviations used are: HTLV, human T-cell leukemia virus; ATL, adult T-cell leukemia; MTI, microtubule inhibitor; MAD, mitotic arrest deficiency; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt; M, mitosis; IL, interleukin; FCS, fetal calf serum; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling.

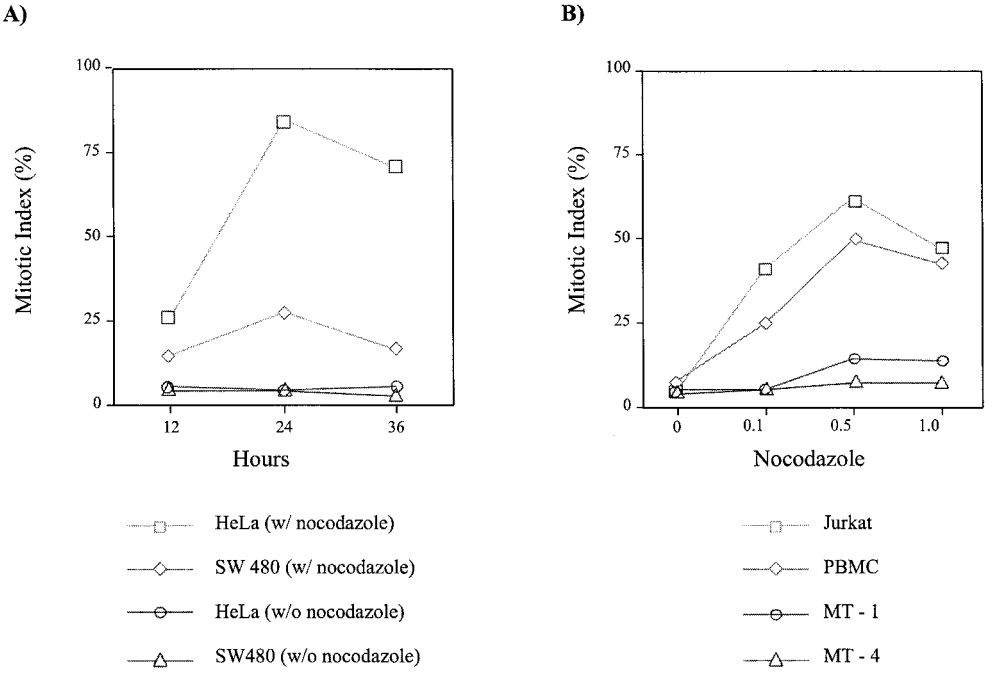


FIG. 1. **Mitotic indices of adherent and suspension cells.** *A*, serial mitotic indices of HeLa and SW480 cells treated with or without 0.5 μM of nocodazole. *B*, dose-titrated response of primary PBMC, Jurkat, and HTLV-I-transformed MT-1 and MT-4 cells to 0.1, 0.5, and 1 μM nocodazole. All values represent averages from three independent assays.

4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) (Dojindo Molecular Technologies, Gaithersburg, MD). Fraction viable cells represent the ratio of WST-8 values from cells treated with drugs relative to that from untreated cells.

Western Blotting—Cells were collected by centrifugation at 1500 rpm, washed in PBS, then resuspended into 0.25 M Tris-HCl. After three freeze/thaws, the cells were centrifuged at 14,000 rpm; the supernatants were saved as the cytoplasmic fractions. The pellets were resuspended into 10 mM Tris-HCl, pH 7.4, sonicated to fragment DNA and were saved as the nuclear fractions. To inactivate potentially infectious HTLV particles, all fractions were adjusted to 0.1% Nonidet P-40. Protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad). Anti-hsMAD2 and anti-hsMAD1 were raised in rabbit to GST-hsMAD2 and GST-hsMAD1 fusion proteins. Mouse monoclonal anti-actin (clone AC-15) was from Sigma. Chemiluminescent immunoblotting was according to manufacturer's procedures (Tropix, Bedford, MA). Visualized bands were detected by scanning and quantified using ImageQuant (Molecular Dynamics, Sunnyvale, CA).

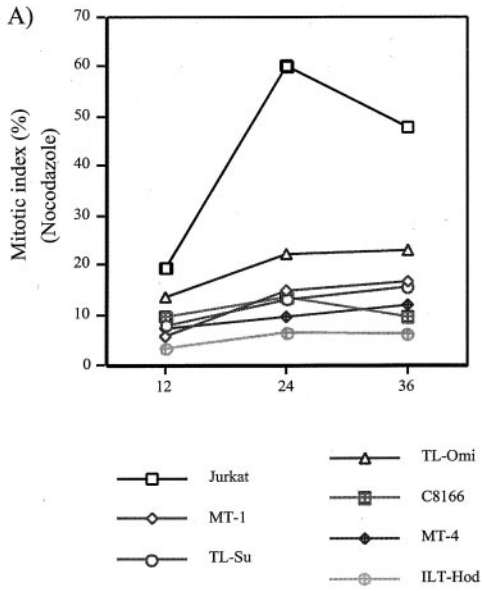
RESULTS

HTLV-I-transformed Cells Are Defective in Mitotic Spindle Assembly Checkpoint—To understand how proliferative dysregulation of ATL cells might correlate with aneuploidy, we investigated mitotic spindle checkpoint function in HTLV-I transformed cells. The mitotic index of cells in response to MTIs (*e.g.* nocodazole or vincristine) reflects the status of this checkpoint. To verify our assays, we first examined two well characterized cancer cells, HeLa (a cervical epithelial cancer) and SW480 (a colon cancer), known to be spindle checkpoint-intact and spindle checkpoint-defective (16), respectively. In our hands, at any given time, 5% of asynchronously propagated HeLa or SW480 cells were in mitosis (M) (Fig. 1*A*). However, when exposed to nocodazole, the two cells responded quite differently. 24 h after treatment, ≥75% of HeLa cells arrested (*i.e.* mitotic index ≥75%) in M-phase (Fig. 1*A*). By contrast, ≥75% of SW480 cells failed to arrest (*i.e.* mitotic index ≤25%) in M and exited mitosis (Fig. 1*A*). These results are consistent with the reported phenotypes for HeLa and SW480. Next, we compared dose-titrated responses of normal peripheral blood mononuclear cells, Jurkat cells (a non-HTLV-I-transformed T-cell leukemia), and two HTLV-I leukemic cell lines, MT-1 and MT-4, to treatment with nocodazole (Fig. 1*B*). PBMCs were first stimulated to proliferate with IL-2 + phytohemagglutinin for 3 days. Subsequently, PBMC, Jurkat, MT-1, and MT-4 cells were separately exposed to nocodazole at final concentrations of 0.1, 0.5, or 1 μM, respectively. 24 h later, cells were assessed for mitotic indices. In the presence of MTI, both PBMC and Jurkat arrested in M, while MT-1 and MT-4 cells did not (Fig. 1*B*). These findings support that the former, but not the latter, cells are spindle assembly checkpoint-intact.

We extended the measurements of MTI-induced mitotic arrest to four additional HTLV-I-transformed T-cells (C8166, ILT-Hod, TL-Omi, and TL-Su; Fig. 2*A*). In our asynchronous cultures, usually between 1.8 and 3.2% of these suspension cells were in M-phase (Fig. 2*B*). Since normal PBMCs were relatively difficult to obtain frequently in large amounts for the many repetitions over which we performed these experiments, and since Jurkat cells had phenotypically intact spindle assembly checkpoint (Fig. 1*B*), we employed the latter cells as positive control. When the HTLV-I and Jurkat cells were treated with 0.5 μM nocodazole and examined serially over time, we found that by 24 h of treatment >60% of Jurkat cells arrested in M (Fig. 2*A*). By contrast, 80–95% of each of the six HTLV cells escaped arrest and exited mitosis (Fig. 2*A*). These results suggest that most, if not all, HTLV-transformed cells may be pervasively defective in the spindle assembly checkpoint.

Spindle Checkpoint-defective Cells Resist MTI-induced Apoptosis—To assess the role of spindle checkpoint on MTI treatment outcome, we measured the apoptotic indices of nocodazole-treated cells. Quantitation of apoptosis based on Hoechst staining and TUNEL assays (data not shown) showed a variance in MTI sensitivity between Jurkat and HTLV cells. At early times after treatment (12 h, Fig. 3*A*), all cells shared similar values. However, by 24–36 h after exposure to MTI, a high extent of apoptosis was observed for Jurkat but not for the HTLV cells (Fig. 3*A*). Thus, when considered together with the data above on mitotic indices, apoptotic sensitivity to MTI correlated inversely with intactness of spindle checkpoint.

Two control experiments excluded that the HTLV cells might



B)

Mitotic indices without nocodazole

	Jurkat	MT - 1	TL-Su	TL-Omi	C8166	MT - 4	ILT - Hod
Mitotic Index	2.94%	3.27%	1.71%	2.91%	1.86%	1.82%	2.04%

FIG. 2. **Comparison of nocodazole-induced mitotic indices in Jurkat and six HTLV-I transformed cell lines.** *A*, mitotic indices of Jurkat and six HTLV-I-transformed cells that were treated as in Fig. 1*B*. *B*, ambient percentages of cells in mitosis from asynchronously propagated cultures. For each of the indicated cell lines, all values represent averages from three independent assays.

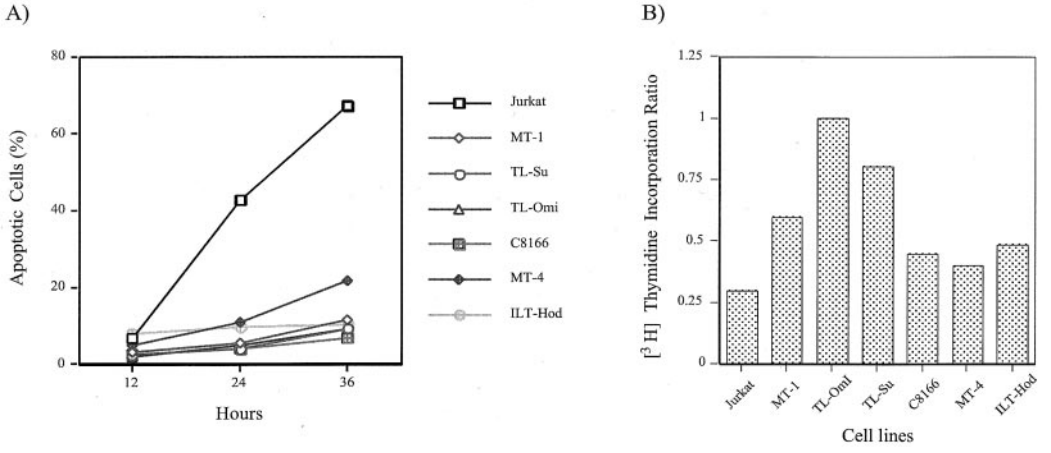


FIG. 3. **Comparison of MTI-induced apoptosis in Jurkat and HTLV-cells.** *A*, serial quantitations over time of apoptosis in the presence of nocodazole (0.5 μM) are shown. Jurkat cells were compared with six HTLV-I-transformed cell lines. *B*, HTLV-I-transformed cells show increased [^{3}H]thymidine incorporation in the presence of nocodazole. [^{3}H]Thymidine was added to nocodazole-treated cells 4 h before harvest. The *bars* represent the ratio of [^{3}H]thymidine incorporation in cells treated with nocodazole for 24 h relative to untreated control cells. Values are averages from two independent assays.

have responded to MTI through non-apoptotic cell death or quiescence. First, we measured [^{3}H]thymidine incorporation by nocodazole-treated cells. Relative to Jurkat, each of the six HTLV cells (Fig. 3*B*) incorporated significantly higher amounts of [^{3}H]thymidine. An interpretation of these results is that nocodazole induced the arrest of Jurkat cells in mitosis (Figs. 1*B* and 2*A*) and that prolonged arrest leads to apoptosis (Fig. 3*A*). On the other hand, because of their spindle checkpoint defect, the HTLV cells were not arrested by MTI and instead progressed unimpeded from M into the next S-phase (*i.e.* DNA synthesis and [^{3}H]thymidine incorporation). Second, using the MTT colorimetric assay for cellular viability, we further quantitated the robustness of growth of cells treated with either nocodazole (Fig. 4*A*) or vincristine (Fig. 4*B*). The MTT findings confirmed the differential viabilities of Jurkat and HTLV cells propagated in the presence of MTIs.

Mislocation of MAD1 and MAD2 Proteins in HTLV Cells—To better understand the reasons for spindle checkpoint loss, we inquired whether the human MAD1 or MAD2 checkpoint proteins might be mutated in the six HTLV cell lines. Full-length cDNAs for MAD1 and MAD2 from Jurkat as well as the six HTLV cells were sequenced. Surprisingly, except for sporadic polymorphic changes, MAD1 and MAD2 cDNAs from all the cells revealed intact open reading frames with no gross deletions or nonsense substitutions.[2] This absence of overt mutation prompted us to consider mechanisms other than gross genetic changes for explaining spindle checkpoint loss in HTLV cells.

We, then, examined the protein expression patterns of MAD1 and MAD2. Intact spindle checkpoint requires nuclear congression of MAD1 and MAD2 (18). Interestingly, in surveying many different ambiently propagated animal cells, we found that in every cell type the great preponderance of MAD1 and MAD2 appeared in the cytoplasm (data not shown). Thus, nuclear migration of MAD1/MAD2 proteins potentially represents a regulatory step in checkpoint function. To ask whether this step might be defective in HTLV cells, we fractionated (by

[2] Y. Iwanaga, unpublished data.

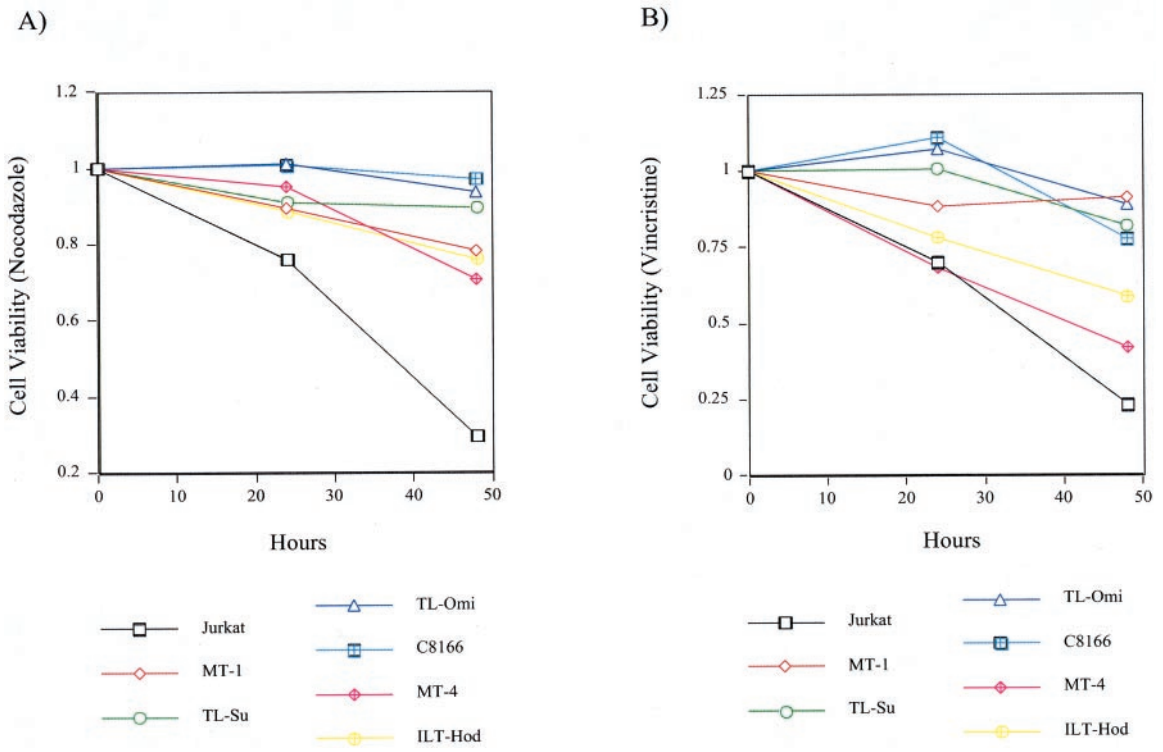


FIG. 4. **Resistance of HTLV cells to vincristine or nocodazole.** Jurkat and six HTLV-I-transformed cell lines were treated with nocodazole 0.5 μM (*A*) or vincristine 0.5 μM (*B*). Samples were taken at 0, 24, 48, and 72 h after treatment. Cell viability represents the ratio of WST-8 dye reduction activity of cells treated with nocodazole or vincristine relative to untreated control cells. Values are averages from three assays.

freeze-thawing) mock-treated and nocodazole-treated cells into insoluble "nuclear" (*N*) and soluble "cytoplasmic" (*C*) portions and probed for MAD1 and MAD2 proteins using specific antisera (Fig. 5). Western blotting revealed that Jurkat cells, both constitutively and in a nocodazole-inducible manner, had higher amounts of nuclear MAD1 and MAD2 (Fig. 5A) when compared with each of the six HTLV-cells. Consistently, the "nuclear/cytoplasmic" (*N*/*C*) ratios for both MAD1 and MAD2 in the HTLV cells were ~50% of that found for Jurkat cells (Fig. 5, *B* and *C*).

Because physical fractionation of cells can be inexact, we sought to confirm the observation of reduced nuclear presence of MAD1 in HTLV cells by immunostaining. Fig. 6 compares the immunofluorescent images of Jurkat and HTLV-I-transformed C8166 cells using monospecific anti-MAD1 serum. In these confocal analyses, the Jurkat cells stained for MAD1 prominently in the nucleus with a punctate distribution (Fig. 6, *top*), while similarly stained C8166 cells showed a nuclear-excluded MAD1 pattern (Fig. 6, *bottom*). Nuclear sparing of MAD1 was also seen in the other five HTLV cells (data not shown).

Enhanced Sensitivity of HTLV-transformed Cells to Combination Therapy with MTI Plus Flavopiridol—In principle, defects in cell cycle checkpoint might be exploited chemotherapeutically to the disadvantage of cancer cells. Here, spindle checkpoint-intact cells (*i.e.* Jurkat) when treated with MTI arrest transiently in M, while checkpoint-deficient cells (*i.e.* HTLV cells) do not arrest and continue into the subsequent G_1. We reasoned that this difference could confer to the latter a heightened sensitivity to a combination of MTI plus a G_1-specific inhibitor, while the former would be protected from G_1 toxicity as a result of MTI-induced M arrest.

To check this reasoning, we treated in parallel Jurkat and HTLV-I-transformed MT4 cells with nocodazole alone, nocodazole followed by flavopiridol, or nocodazole simultaneous with flavopiridol (Fig. 7). Cellular viabilities were assessed by MTT assay. Jurkat cells, as expected, were more sensitive than MT4 cells to nocodazole alone (Fig. 7*A*). However, opposite relative susceptibility profiles were seen when a G_1-potent inhibitor, flavopiridol (19), was added to nocodazole (Fig. 7, *B* and C). Checkpoint-defective MT4 cells exposed to either nocodazole followed by flavopiridol or nocodazole simultaneous with flavopiridol were significantly less viable than checkpoint-intact Jurkat cells. These effects relate to spindle checkpoint status and are independent of p53 activity, since both Jurkat (20) and HTLV-I-transformed cells lack p53 function (21).

DISCUSSION

Cancer cells differ from normal cells at multiple genetic loci. In evolving from a normal to a cancerous state, cells lose some or many of the biological checkpoints which monitor the fidelity of DNA replication, repair, and segregation (22). In different cancers, different losses in cell cycle control appear to be emphasized. For instance, most breast cancers have mutated p53 or pRb, leading to a defective G_1/S control (23), while most colorectal cancers (85%) have lost the ability to censor against aneuploidy (24). Here, we report that ATL cells are prevalently defective in the spindle assembly checkpoint that monitors for fidelity of chromosomal segregation during mitosis.

What are some implications of checkpoint loss? A practical one applies to the treatment of cancers. Most current anticancer drugs have low therapeutic indices (25) (*i.e.* toxic dose per therapeutic dose). *In vivo*, chemotherapy of tumors is limited by drug toxicity for normal cells. Molecular differences in checkpoint functions between neoplastic and non-neoplastic tissues potentially permit selective drug designs that could target cancers while sparing normal counterparts. For example, initial results from model cell systems had encouraged the idea that loss of p53 (26) or p21 (27) proteins might potentiate cellular sensitivity to MTIs such as taxol and vincristine. Unfortunately, this was subsequently called into question by difficulties in reproducing enhanced MTI susceptibility in *bona*

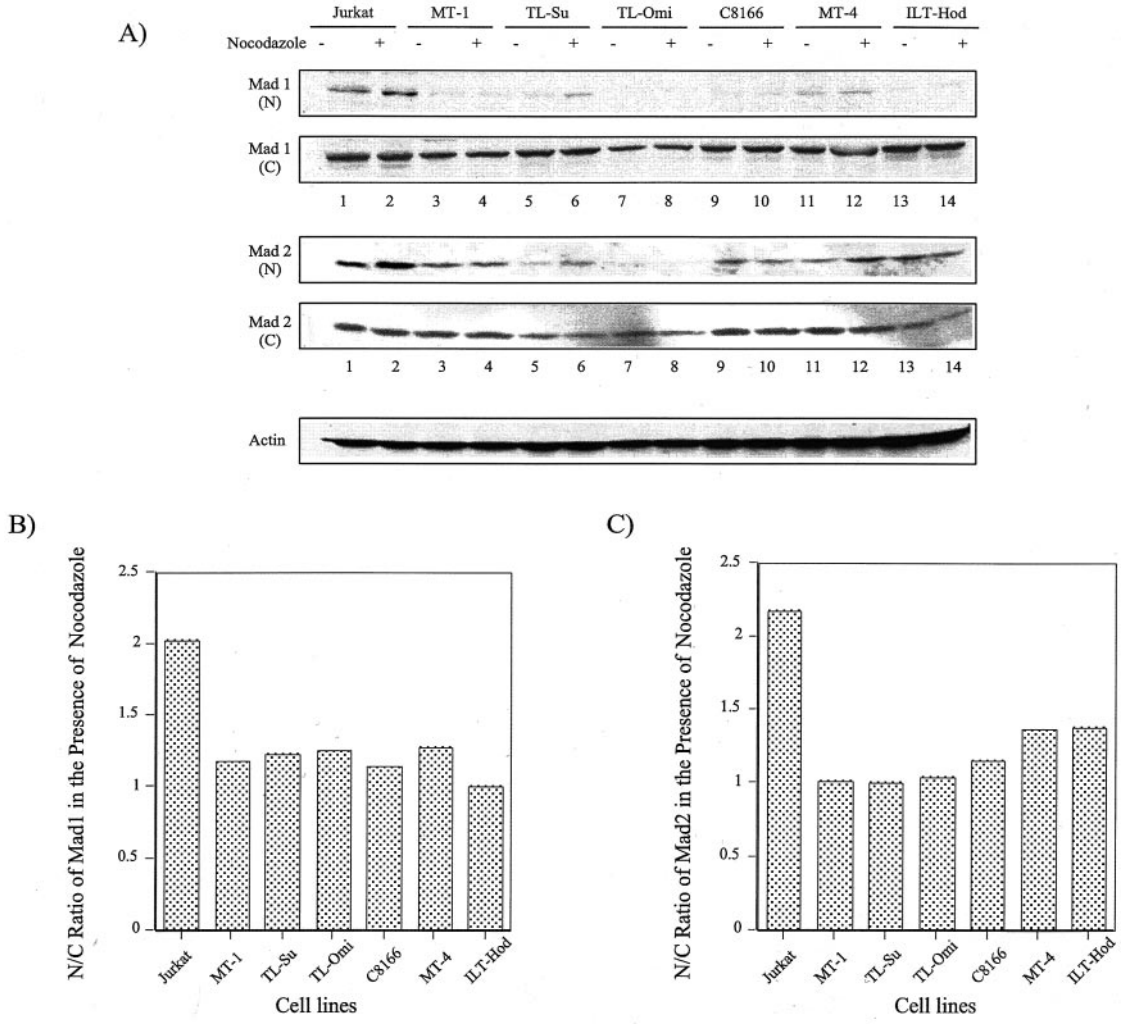


FIG. 5. **Reduced presence of MAD1 and MAD2 in the nuclear fraction of HTLV-I-transformed cells.** *A*, Western blotting of cytoplasmic and nuclear extract using antisera to either MAD1 or MAD2. *N* and *C* represent nuclear and cytoplasmic fractions, respectively, as defined by pelletable and soluble fractions after three freeze-thaws of cells. Actin (*bottom*) signals provide controls for equivalence of sample loading. Reduced nuclear/cytoplamic ratios of MAD1 (*B*) and MAD2 (*C*) after treatment with nocodazole in HTLV-I-transformed cells compared with Jurkat cells were seen. MAD1/actin and MAD2/actin ratios were calculated first, then nuclear/cytoplasmic ratios were derived. The *bars* represent the N/C ratios in cells treated with drugs relative to untreated control cells.

fide human cancers (28, 29). Further exacerbating the confusion were reports that loss of p53 increased (30) and decreased (31) sensitivity to DNA-damaging agents. It, thus, remains unclear how p53 defects in cancers might be used to guide the chemotherapy of tumors.

The spindle assembly checkpoint monitors integrity of chromosomal segregation during mitosis (12, 31, 32) in a p53-independent manner. This checkpoint, activated in cells by exposure to MTIs, plays a central role in guarding against the emergence of aneuploidy. Current thinking assumes two links between the spindle checkpoint and oncogenesis: 1) defective spindle checkpoint is frequent in cancers (33), and 2) defects arise from mutations in one of seven known spindle checkpoint genes (12). Interestingly, a recent study has questioned the presumed prevalence of spindle checkpoint loss in cancers (17), and intensive searches for mutations in the spindle checkpoint genes have so far shown such changes to be exceedingly rare in human tumors (16, 34–37). Hence, three questions remain to be clarified. What is the true nature of the spindle checkpoint in human cancers? How could frequent checkpoint loss be reconciled with the rarity of mutations in checkpoint genes? And if spindle checkpoint functions differ between normal and cancer cells, could this difference, in analogy to the thinking with

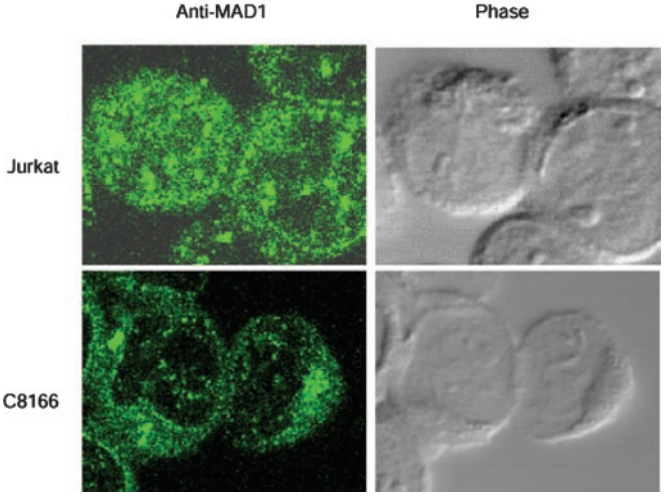


FIG. 6. **Immunostaining of MAD1 in Jurkat and HTLV-I transformed C8166 cells.** Methanol-fixed cells were incubated with monospecific anti-MAD1 serum and visualized using a Carl-Zeiss confocal microscope. Fluorescent images are shown at the *left*; phase contrast images are shown at the *right*.

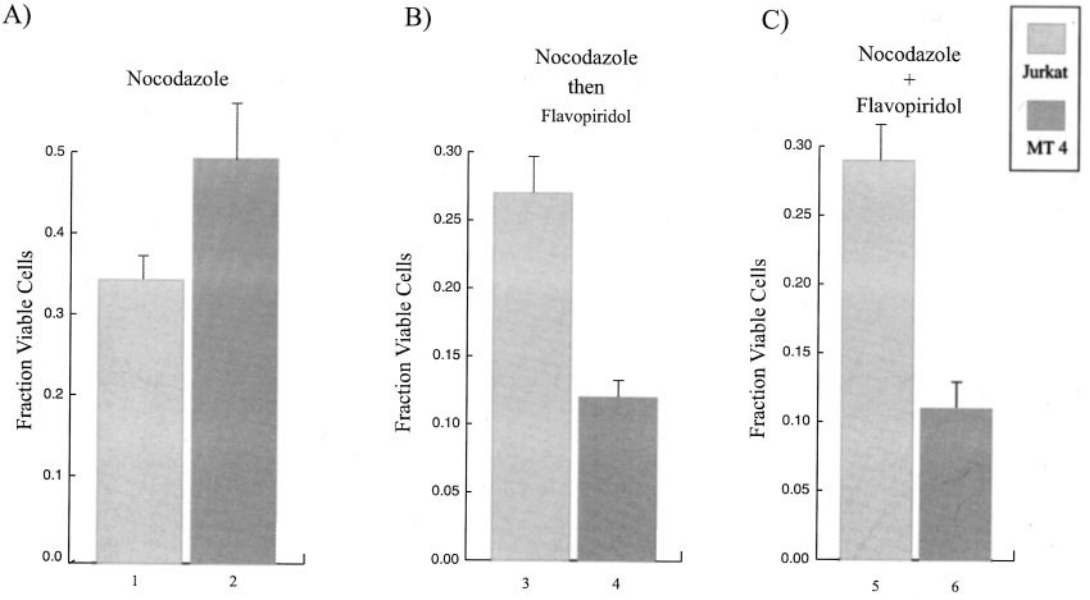


FIG. 7. **Different relative susceptibilities of Jurkat and HTLV cells to nocodazole alone or nocodazole with flavopiridol.** Cells were treated in several ways: mock-treated for 48 h, 0.5 μM nocodazole alone for 48 h, 0.5 μM nocodazole for 12 h and then 300 nM flavopiridol for 36 h, and 0.5 μM nocodazole + 300 nM flavopiridol for 48 h. The viability of cells was measured by a modified MTT dye reduction assay using WST-8. Fraction viable cells represents the ratio of WST-8 value from treated cells divided by the counterpart value from mock-treated cells. Data are averages from three independent assays.

p53, play a role in the clinical response of cancers to chemotherapeutic agents?

Our findings here from HTLV-I-transformed cells begin to address the above questions. In studying six ATL cell lines, we found that 100% of the samples were defective in the mitotic spindle checkpoint. This suggests that loss of this M function is common, if not universal, in ATLs and argues that a similar defect could contribute to other tumors. Intriguingly, in HTLV-I cells, checkpoint loss does not require mutation in checkpoint genes. In these cells a not yet understood mechanism, which mislocates MAD1 and MAD2 proteins into the cytoplasm (Fig. 5 and 6), appears to explain loss-of-function. Cytoplasmic sequestration has also been reported for p53 in breast cancers (38). This type of mechanism potentially reconciles the paradox of frequent spindle checkpoint loss in cancers unaccompanied by mutations in checkpoint genes (16, 34–37).

In good agreement with our findings of mitotic checkpoint loss, in a review elsewhere of 107 ATL cases, Kamada *et al.* (8) found all HTLV-I leukemic samples to be karyotypically abnormal. We have independently verified Kamada's conclusion of aneuploidy in ATL cells by direct cytogenetic examination of some of the HTLV-I cell lines used here (data not shown). For example, our MT-1 cell line showed a modal chromosome number of 83, and our TL-Omi cells had a modal number of 45. The TL-Su cells existed as two populations, one having a modal number of 48 chromosomes and a second having 94 chromosomes. By contrast, our particular Jurkat sample was largely tetraploid with a modal chromosome number of 92. Mechanisms for generating aneuploidy and polyploidy are not the same (39). Our cytogenetic results are consistent with HTLV-I cells being defective in the mitotic spindle checkpoint and Jurkat cells being lost in a different checkpoint, which guards against polyploidy.

From a practical perspective, the prevalent M spindle checkpoint loss in ATLs is both daunting and potentially useful in structuring cancer chemotherapy. Current ATL treatments are challenging and largely ineffective (40). Projected 4-year survival rates for acute- and lymphoma-type ATLs stand at 5 and 5.7%, respectively (41). Our findings here offer some insight into the chemoresistance of ATL cells. Thus, we can explain for the first time why MTIs unto themselves have no apoptosis-inducing capacity and are of little therapeutic value for ATLs. Considering this mechanistic explanation and the empirically dismal treatment outcomes as well as potentially significant neuro and myeloid toxicities (42), one may wish to re-examine the use of MTIs (*e.g.* vincristine) as a standard component of most ATL chemotherapy (43). On the other hand, our study does reveal that judicious combination of MTI with a G_1 toxin could spare M-arrested normal cells while killing selectively non-arrested ATL cancer cells (Fig. 7). This aspect of spindle checkpoint loss merits further investigation and could potentially be important in the design of future cancer drugs that exploit this cellular phenotype.

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